Effect of Pasteurization and Evaporation on Foot-and-Mouth Disease Virus in Whole Milk from Infected Cows

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ABSTRACT

The effects of pasteurization and evaporation on foot-and-mouth disease virus in whole milk from infected cows obtained one day postinoculation were studied. Virus survived the heating of milk at high temperature-short time pasteurization at 72°C for 15-17 seconds. In addition, virus from infected milk survived heating at 80°C for the same time. Infective virus also survived in the pasteurized milk after evaporation at 65°C to 50% of the original volume. The bovine udder was found to be highly susceptible to foot-and-mouth disease virus replication. Seven $\log_{10}$ plaque-forming units/ml of virus were recovered in whole milk 24 hours postinoculation, and decreasing titers were recovered for as long as seven days postinoculation.

RÉSUMÉ

Cette expérience visait à étudier les effets produits sur le virus de la fièvre aphteuse par la pasteurisation et l'évaporation du lait entier de vaches infectées depuit 24 heures. Le virus résista au chauffage du lait à 72°C, durant 15 à 17 secondes; il survécut également à un chauffage à 80°C, et de même durée. Il survécut aussi dans du lait d'abord pasteurisé et ensuite évaporé à 50% de son volume initial, à la température de 65°C. Le pis de la vache s'avéra un milieu très favorable à la multiplication du virus de la fièvre aphteuse. Les auteurs en recouvrèrent une quantité équivalente à 7 $\log_{10}$ unités formatrices de plages/ml de lait entier, 24 heures après l'infection; ils en retrouvèrent aussi en concentration décroissante, durant les six jours ultérieurs.

INTRODUCTION

Recovery of significant quantities of foot-and-mouth disease virus (FMDV) in milk from cows before FMD infection becomes evident (6, 15, 21) strongly suggests a method for wide scale dissemination of the virus. Thus, the effectiveness of pasteurization and evaporation techniques on the inactivation of FMDV in whole milk and some milk products is of the utmost concern. The recommended minimum temperatures for the pasteurization of milk (61.7°C for 30 minutes or 71.1°C for 15 seconds[1]) are adequate for inactivation of many viruses (13, 16, 17, 20); however, in various other test systems (1, 3, 4, 5, 11, 18, 19), these and even higher temperatures are not satisfactory for inactivating FMDV.

Sellers (19) observed traces of FMDV in milk exposed to heat at 85°C for five seconds. Nikitin and Vladimirov (18) found that FMDV persisted in dried skim milk

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[1]Grade "A" Pasteurized Milk Ordinance, 1965. Recommendations of the United States Public Health Service.

for periods up to two years after contaminated dried milk powder was heated at high temperatures.

The heat resistance of FMDV in non-milk mediums has been documented extensively (1, 3, 4, 5). In 1959, Bachrach (3) showed that infectious ribonucleic acid (RNA) could be recovered from FMDV boiled at 100°C for five minutes.

Previous studies on the resistance to heat of FMDV in milk have all entailed the addition of virus to milk before testing. However, in the work reported here, whole milk collected from FMDV-infected cows before the appearance of clinical signs of FMD was subjected to high temperature-short time (HTST) pasteurization as well as pasteurization followed by evaporation. The heat treated milk was assayed for infectivity in cell culture and cattle.

MATERIALS AND METHODS

CATTLE

Three grade dairy cows used in the study were obtained from commercial sources. Their ages varied from three to seven years. All had subclinical mastitis when received. The steers used for testing the infectivity of the processed milk were grade white-faced Herefords, approximately 15-18 months old. The housing and mainteance of the cattle were described previously (8, 12).

VIRUS

The FMDV used in this study was type A, subtype 3 (A_3), strain Mecklenburg, originally isolated in Germany during an outbreak in 1944 and received at the Plum Island Animal Disease Center in 1971 as the sixth passage in bovine tongue epithelium. This material was passaged twice in cattle by intradermalingual (IDL) inoculation.

After appearance of lesions, tongue epithelium was harvested, ground with a mortar and pestle and centrifuged. The supernatant fluid was removed, and antibiotics were added at the rate of 100 units penicillin, 100 μgm streptomycin sulfate and 50 units mycostatin/ml of fluid. The final inoculum consisted of a mixture of equal volumes of virus suspension and Hanks' balanced salt solution containing 0.5% lactalbumin hydrolysate (HLH).

ANIMAL HANDLING AND INOCULATION

When the cows were received in the isolation area, they were examined clinically for mastitis, and their temperatures were recorded. The mammary glands were washed with warm water containing 0.02 ppm Wescodyne solution[2] and rinsed with warm water. The ends of the teats were swabbed individually with 70% ethyl alcohol. Samples were obtained from each quarter and inoculated in blood agar and crystal violet blood agar plates. Blood for serum extraction was collected from the jugular vein. Two ml (ca 7.0 $\log_{10}$ plaque-forming units [PFU]/ml of the virus preparation) was then inoculated into the milk sinus of the right front teat and 2.0 ml into the left rear teat. In addition, 2.0 ml of the same suspension was inoculated intravenously into the jugular vein.

A milk sample from the first cow was collected four hours postinoculation and at 22 and 26 hour intervals thereafter for seven days. Milk samples from the second and third cows were collected at four hours postinoculation and after that at 24 hour intervals.

DETECTION OF VIRUS NEUTRALIZATION ACTIVITY

Foot-and-mouth disease virus, A_3 specific neutralizing activity in bovine sera was measured by the mixing of 100 mouse 50% infective doses (ID_{50}) of type A_3 FMDV with each dilution of a dilution set of the test serum and testing each serum-virus mixture for unneutralized virus by inoculation of suckling mice (12).

PASTEURIZATION PROCEDURES

A laboratory size pasteurizer was prepared as follows: approximately nine liters

[2]Polyethoxy polypropoxy ethanol-iodine complex; monophenyl ether of polyethylene glycol-iodine complex containing 1.6% titratable iodine, West Chemical Co., New York, New York.

of distilled deionized water (50°C) was added to a 20 liter heavy-walled glass water bath. Pasteurization temperatures (72°C and 80°C) were reached and maintained by use of glass-type immersion heaters.[3] A metal rack was placed in the water bath to hold the sample tubes so that the level of water in the bath would be above that of the milk samples. The water bath was placed on a stainless steel frame positioned immediately above a large magnetic mixer.

Ten ml volumes of chilled (12-14°C) milk were placed in each of five 125 x 20 mm glass tubes containing a small magnetic mixing bar. A thermometer was placed in one tube to monitor temperatures. When the milk sample in the monitor tube reached the desired temperature, the tubes were held at this temperature for the exposure period of 15-17 seconds, then they were immediately immersed in an ice bath (4°C). When the temperature reached 20°C, the milk samples were pooled and held in an ice bath at 4°C. The process was repeated in order to obtain a total volume of 100 ml of pasteurized milk.

A part of the sample was assayed for infectivity in primary bovine kidney (BK) cell culture as described previously (2). The phosphatase test (14) was used to determine whether pasteurization was complete.

Evaporation Procedures

The milk used in the evaporation procedure consisted of infected milk samples collected 24 hours postinoculation, pasteurized and stored at –20°C for seven days. Before use, the samples were thawed in a 37°C water bath. The milk was decanted aseptically into a 250 ml filtration flask fitted with a canalized rubber stopper that allowed for the placement of a heat-sensitive probe (Tele-thermometer)[4] in the milk. The flask was placed in a heated water bath as previously described. The samples of pooled, pasteurized milk were reduced to 50% of their original volume by heating at 65°C under a vacuum of 60 centimeters of mercury for an average time of 64.5 minutes.

[3]Vycor, Corning Glass Works, Corning, New York.

[4]TNTronics, New Paltz, New York.

Detection of Virus

A part of the evaporated sample was assayed in cell culture as described in "Pasteurization Procedures". The other part was inoculated into a steer, 2.0 ml IDL and 35 ml intramuscularly. The animals were observed daily for vesicular lesions; when these developed, samples of vesicular material were harvested for complement fixation (9). Steers that remained free of clinical signs of FMD for 14 days postinoculation were subsequently inoculated IDL with 2.0 ml containing 7 $\log_{10}$ PFU/ml of FMDV A_3 (Mecklenburg).

Assay of Samples in Cell Cultures

Serial tenfold dilutions of the processed and raw milk samples were prepared in chilled HLH, and 0.1 was adsorbed onto each of three BK cell cultures (2).

RESULTS

Recovery of Virus

Untreated Milk Samples — Animals developed vesicular lesions three, four and seven days postinoculation (Table I). None of the sera studied had neutralizing antibodies against FMDV before the studies. Virus was replicated to significant titers in the mammary gland in the presence of *Streptococcus agalactiae* infection; the highest titers were found one day postinoculation and then decreased for seven more days, when it was no longer detectable (Table I). In two of the three animals, a second peak of virus infectivity was found in the milk four to five days postinoculation.

After Pasteurization — The effect of pasteurization at 72°C and 80°C for 15-17 seconds was studied. At least 2 $\log_{10}$ PFU/ml of virus was recovered in samples pasteurized at 72°C for 15-17 seconds, and 1.0 $\log_{10}$ PFU/ml in samples pasteurized at 80°C (Table II).

After Evaporation — Virus was not detected in the pasteurized evaporated milk samples when assayed in cell culture; however, when similar samples were inoculated into steers, vesicular lesions developed within two days.

DISCUSSION

The heat inactivation of FMDV and its nucleic acid has been described in considerable detail (1, 3, 4, 5). In a study conducted in 1960 by Bachrach (4), a small fraction of the virus population resisted inactivation by heating at 55°C for one hour. Sellers (19) found that in an alkaline pH, the survival of the virus exposed to HTST pasteurization is enhanced significantly. In the studies reported here, the mean pH of the milk samples collected one day postinoculation was 7.15 ± standard deviation, a pH that according to Sellers would favor survival of the virus.

TABLE I. Concentration of Virus in Whole Milk Samples Collected After Intramammary and Intravenous Inoculation of Cows with Foot-and-mouth Disease Virus

Days Postinoculation	Log_{10} PFU/ml Cow No. 1	Cow No. 2	Cow No. 3
0	0.0	0.0	0.0
0.16	1.9	3.2	1.9
1	7.0	7.5	6.7
2	5.1	4.1	3.6
3	5.3	4.5	4.3[a]
4	5.1[a]	5.8	4.7
5	3.4	5.2	5.2
6	3.5	3.1	4.6
7	0.0	3.4[a]	1.4
8	0.0	0.0	0.0

[a]Appearance of vesicular lesions

The effect of minimum HTST pasteurization and evaporation procedures on inactivation of FMDV in whole milk during the preclinical stage of the infection was studied. In contrast to the studies by Nikitin and Vladimirov (18) and Sellers (19), virus was not added to milk but was secreted into the milk by experimentally infected cows.

The recovery of 7 log_{10} PFU/ml of virus in whole milk collected one day postinoculation was 100-fold greater than that reported in infected milk from cows in disease outbreaks (15). This finding might suggest that the initial concentration of virus inoculated into cows in this study was too high. However, as further evidence for the resistance of FMDV to pasteurization and evaporation, in studies currently underway on the survival of FMDV in skim milk containing 5 log_{10} PFU/ml of FMDV, the virus was not completely inactivated after pasteurization and evaporation. However, complete pasteurization of all samples was confirmed by a negative phosphatase test.

In a country where FMD is enzootic, most of the cattle would have circulating antibodies as a result of the disease or routine vaccinations. In addition, milk from such animals is known to contain neutralizing antibodies (7) that would undoubtedly influence virus that might be detected in the milk. On the other hand, Burrows *et al* (7) have shown that virus is secreted in milk during the prodromal stage of the disease in infected and convalescent animals. During such outbreaks of the disease, milk delivered to a processing plant is likely to

TABLE II. Heat Inactivation of Foot-and-mouth Disease Virus in Milk from Infected Cows One Day Postinoculation

Cow No.	Temperature[a]	Virus concentration in cell cultures[b] Before pasteurization	After pasteurization	Pasteurized evaporated[c] milk samples inoculated in[d] Cell culture	Cattle
1	72°C	6.9	1.6	−	+
	80°C	6.9	0.91	−	+
2	72°C	7.5	2.0	−	+
	80°C	7.5	2.1	−	+
3	72°C	6.7	3.0	−	+
	80°C	6.7	2.0	−	+

[a]Temperature of pasteurization (15-17 seconds)
[b]Log_{10} PFU/ml
[c]Evaporation temperature and time was 65°C for 64.5 minutes
[d]− no positive reaction; + positive reaction

contain large quantities of virus from cattle with little or no antibodies, and the likelihood of these circumstances are of concern to FMD-free countries importing dried milk and other dairy products.

In view of the above studies and those of Dawson (10), Hedger and Dawson (15), Sellers (19) and Terbrüggen (21), transmission of FMDV in milk from an infected cow after pasteurization and evaporation is a definite possibility and should be of much concern to animal health authorities in countries free of the disease.

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